Review Article

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Haemophagosytic limphohistiosytosis (HLH) in adults - clinical features, triggering diseases, prognostic factors and outcomes: Report of thirty-three cases

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Objective: To analyse clinical features, triggering diseases, treatment strategies and prognostic factors in patients with secondary haemophagosytic limphohistosytosis (SHLH).

Methods: We retrospectively analysed thirty-three patients with positive haemophagocytosis bone marrow biopsies, all collected between 1995 and 2015 from two different hospitals.

Results: The average age was 44.39 years with a man/women ratio 1.06/1. The underlying diseases were as follows: Autoimmune diseases (n=11), liver or kidney transplant (n=9), haematological malignancies (n=5) infection (n=5) and solid organ cancer (n=3). The average time from hospitalisation to death was 49.95 days (49.95 ± 39.608). Three different prognostic factors were separately analysed: overall mortality, severe disease (less than two months to death) and extremely severe disease (less than one month to death). Risk factors associated to overall mortality were age >35 years ($p<0.011$), severe cytopenias such as anaemia ($p<0.002$), bicytopenia ($p<0.007$) and pancytopenia ($p<0.025$), ongoing lung involvement ($p<0.012$) and sepsis ($p<0.044$). Risk factors for severe disease were underlying treatment with corticosteroids alone ($p<0.013$), severe anaemia ($p<0.002$), neutropenia ($p<0.027$) and pancytopenia ($p<0.016$). Severe hypofibrinogenemia ($p<0.039$), ongoing lung involvement ($p<0.022$) and a late start to specific treatment ($p<0.047$) were highly indicative for severe disease, whereas underlying autoimmune disorder ($p<0.003$) and the simultaneous use of immunosuppressant at the onset ($p<0.007$) seemed to act as a protective factor for severe disease. Risk factors for extremely severe disease were underlying solid organ cancer ($p<0.043$), severe cytopenias such as thrombocytopenia ($p<0.016$), bicytopenia ($p<0.016$) and pancytopenia ($p<0.009$), and a late start to specific treatment ($p<0.043$).

Conclusions: Patients with secondary HLH might have a different prognosis according to the triggering disease. Underlying autoimmune disorder might be related to a better prognosis and malignancy might indicate high mortality. Early recognition and specific treatment is essential for the patient's survival whereby tight suspicion is necessary for an attempt at curative therapy to be made.

Keywords: haemophagosytic lymphohistiosytosis • macrophage activation syndrome • haemophagositosis • autoimmune diseases • prognostic factors • adults

Introduction

Haemophagocytic lymphohistiosytosis (HLH) is the clinical manifestation of a wide array of different entities, which include primary or familial haemophagocytic lymphohistiocytosis (FHLH) and secondary forms. The hallmark is haemophagocytosis, appearance of activated macrophages that have engulfed other haematopoietic elements. FHLH, mainly documented in early infancy, is related to familiar inheritance or genetic causes [1]. Secondary forms (SHLH), also called reactive HLH, are frequently diagnosed in adults and refer to cases with underlying infection, malignancy or autoimmune disease [2]. Over the last decade immunosuppression, immunodeficiency, auto inflammatory diseases and inborn errors of metabolism have been also described as triggering diseases [3]. Macrophage activation syndrome (MAS) is a secondary form, recently reported in patients with autoimmune or auto inflammatory diseases, especially patients with systemic juvenile idiopathic arthritis (SJIA), Systemic lupus erythematosus (SLE), and adult onset systemic Still's disease (AOSD) [4]. Our study reports 33 adult cases and we try to analyse

triggering diseases, prognostic factors, treatment strategies and outcomes.

Patients and methods

This study includes thirty-three patients from two hospitals, Doce de Octubre, Madrid and Marina Salud, Denia.

All patients were diagnosed positive for haemophagocytosis by bone marrow biopsy reviewed by expert pathologists between 1995 and 2015. Patients without positive biopsy were not included. All patients were diagnosed with HLH according to HLH-diagnostic guidelines during the time of their hospitalisation. We also collected the following chart for each patient: age, gender, underlying triggering disease or infection, underlying treatment with corticosteroids, immunosuppressant or mycophenolate mofetil (MFM). Clinical features such as long-lasting fever, splenomegaly, ongoing liver, central nervous system (CNS) or lung involvement, and sepsis were also reported. Underlying and ongoing infection were separately evaluated. The laboratory data collected from each patient were: haemoglobin, neutrophil and platelet count, as well as ferritin, fibrinogen and triglyceride levels, and NK cell counts (if available). We separately evaluated three outcomes: overall mortality (OM), severe disease (SD) (less than two months to death) and extremely severe disease (ESD) (less than one month to death). Quantitative variables such as age were evaluated by T Student test for simple independent variables. For the rest of the qualitative variables we used Chi square test. Statistical significance was set at $P<0.05$. Associations were expressed using Odds ratios (ORs) with 95% confidence interval (95% CI).

Results

This study includes thirty-three patients from two hospitals, Doce de Octubre, Madrid and Marina Salud, Denia.

All patients were diagnosed positive for haemophagocytosis by bone marrow biopsy reviewed by expert pathologists between 1995 and 2015. Patients without positive biopsy were not included. All patients were diagnosed with HLH according to HLH-diagnostic guidelines during the time of their hospitalisation. We also collected the following chart for each patient: age, gender, underlying triggering disease or infection, underlying treatment with corticosteroids, immunosuppressant or mycophenolate mofetil (MFM). Clinical features such as long-lasting fever, splenomegaly, ongoing liver, central nervous system (CNS) or lung involvement, and sepsis were also reported.

Clinical features and underlying diseases

The average age was 44.39 years (range 18-78 years) Table 1. 17 (51.5%) patients were male and 16 (48.5%) were female, with male/female ratio 1.06/1. 11 (33.3%) patients were diagnosed as having autoimmune disease at the onset, 9 (27.3%) had liver or kidney transplant, 5 (15,2%) haematological malignancies, 5 (15.2%) underlying infection and three (9.1%) solid organ cancer. 23 (69.7%) patients had infection at the onset and in five of those patients the infection was the only known trigger, while for the rest infection and other triggers were simultaneously presented. 17 (51.5%) patients received corticosteroids at the onset, but only six of them had long-term treatment with corticosteroids alone. 20 (60.6%) patients received any immunosuppressant at the onset (including corticosteroids). 10 (90.9%) of the 11 patients with autoimmune disorder had immunosuppressant at the onset and 5 corticosteroids alone. The autoimmune disorders collected in our study were: systemic lupus erythematosus (SLE) (n=5), rheumatoid arthritis (RA) (n=3), adult onset systemic Still's disease (ASD) (n=2) and Sjögren´s syndrome (SS) (n=1). Haematological malignancies were: Non-Hodgkin Lymphoma, 2 patients, T cell lymphoma, 2 patients and Myelodysplastic syndrome, 1 patient. The most frequent infectious agents were *Epstein Barr virus* (EBV) (n=8), *Mycobacterium tuberculosis* (n=2), Leishmania (n=2), Cytomegalovirus (CMV) (n=2), *Toxoplasma gondii* (n=2), *Aspergillus fumigatus* (n=3), *Staphylococcus aureus* (n=1), *Human Herpesvirus* type 6 (n=1), *Pseudomonas aeruginosa* (n=1) and HIV (n=1). All patients had long-lasting fever. Splenomegaly was present in 24 (72.7%) patients and liver dysfunction in 19 (59.4%). Central nervous system (CNS) manifestations such as convulsions had 2 patients, meningoencephalitis 3, encephalopathy 6 and 2 had cephalea. 17 (51.5%) patients developed pulmonary disorders during the disease course, such as pneumonia (n=7), pleural effusions (n=5) and acute respiratory failure (n=5). Severe haemorrhage was reported in 4 patients, significant skin rash in 1 patient and arthralgia in 4. Severe ongoing infection or sepsis was developed by 6 patients.

Laboratory tests

All values were fixed at the time of diagnosis.

Table 1. Clinical features and underlying diseases

No.	Age	Gender	Underlying disease	Ongoing infection	Glucocorticoids at the onset	Immunosuppressant at the onset	Treatment set	Outcomes	
								Dead (days)	Alive (years)
1	60	Female	SS	-	+	-	4	70	-
2	47	Male	HIV infection	-	-	-	4	44	-
3	61	Male	Kidney transplant	*St. aureus*	-	+	2	21	-
4	18	Female	VEB infection	-	-	-	3	28	-
5	17	Male	VEB infection	-	-	-	4	44	-
6	38	Female	VEB infection	-	-	-	3	-	>10
7	25	Male	T-cell lymphoma	VEB	-	-	4	40	-
8	16	Female	SLE	Herpes virus 6	+	+	4	-	>5
9	65	Female	Rectal cancer	-	-	-	1	14	-
10	46	Male	Disseminated tuberculosis	-	-	-	4	-	>3
11	56	Female	Pituitary carcinoma	Tuberculosis	-	-	1	14	-
12	54	Female	Liver transplant	VEB	+	+	2	-	>10
13	48	Male	Liver transplant	Leishmania	-	-	3	70	-
14	62	Male	Liver transplant	CMV	+	+	3	44	-
15	62	Male	Liver transplant	-	+	+	1	44	-
16	25	Male	Non-Hodgkin lymphoma	-	-	-	3	-	>3
17	31	Female	SLE	-	+	-	3	-	>2
18	63	Male	Kidney transplant	*Toxoplasma gondii*	+	+	2	21	-
19	62	Male	Liver transplant	VEB	+	+	2	40	-
20	78	Female	Cutaneous T-cell lymphoma	CMV	+	+	4	90	-
21	37	Male	Kidney transplant	-	+	+	2	-	>4
22	26	Female	RA	-	+	+	2	-	>3
23	43	Female	Kidney transplant	VEB	-	+	2	-	>4
24	19	Female	AOSD	-	+	-	4	-	>5
25	30	Female	T-cell lymphoma	VEB	-	-	4	21	-
26	30	Male	Myelodysplastic syndrome	*Toxoplasma gondii*	-	-	4	60	-
27	70	Male	RA	*Aspergillus fumigatus*	+	+	3	14	-
28	73	Male	RA	-	+	-	2	90	-
29	43	Male	SLE	Leishmania	-	+	2	-	>1
30	31	Female	SLE	*Aspergillus fumigatus*	+	+	3	-	Lost follow up
31	28	Female	AODS	-	+	-	3	-	>1
32	50	Male	Squamous cell oral carcinoma	*Pseudomonas aeruginosa*	+	-	2	-	>1
33	51	Female	SLE	*Aspergillus fumigatus*	-	-	3	180	-

AOSD- adult onset systemic Still's disease, RA- Rheumatoid arthritis, SS- Sjögren´s syndrome, SLE- systemic lupus erythematosus, VEB –virus Epstein-Barr, CMV- cytomegalovirus
F= female, M=male

Haemoglobin (Hgb) levels were available in all patients, with median haemoglobin nadir 84 +/- 1.703 g/L (range 29.0-139.0). 19 (57.6%) patients had severe anaemia (Hg<to 85 g/L), 13 (39.4%) had severe neutropenia (neutrophils <0.5 × 10*9/L) and 15 (45.5%) had severe thrombocytopenia (platelets <35 × 10*9/L). 9 (27.3%) patients had severe bicytopenia such as anaemia and neutropenia, and 11 (33.3%) had anaemia with thrombocytopenia. Severe pancytopenia was detected in 7 (21.2%) patients. 16 (48.5%) patients had high triglyceride levels (TG>265 mg/dL). Hypofibrinogenemia (<1.5 g/L) was detected in 10, and severe hypofibrinogenemia (<1.0 g/L) in 6 patients. NK cell count was available in 8 patients. 7 of

them had a very low NK cell count (range 0-10) and one had normal NK values (99). Ferritin levels were available in 19 patients. 16 of those patients had ferritin levels higher than 500 ng/mL, and 5 had ferritin levels higher than 5000 ng/mL (Table 2).

Treatment

In order to evaluate the outcome of the used therapies we divided the patients into 4 sets;

- Patients without specific treatment, 3 (9.1%).
- Corticosteroids alone, 10 (30.3%).
- Corticosteroids with another immunosuppressant (and immunoglobulin in 3 cases), 10 (30.3%).
- Corticosteroids, cyclosporine and Etoposide, 10 (30.3%) patients.

Two patients with NHL (Non-Hodgkin Lymphoma) also received Cyclophosphamide, Vincristine and Adriamycin, and another two patients who had T cell lymphoma received Rituximab. Antibiotics were administered in all cases with bacterial or mycobacterial infection (n=15) and Acyclovir in eight patients. Three patients received intravenous immunoglobulin. The immunosuppressant drugs widely used were cyclosporine, 11 patients, tacrolimus 4, and azathioprine 1 patient.

Bone marrow biopsy

Table 2. Laboratory tests

Levels fixed at the time of diagnosis							
Patient	**Haemoglobin g/L**	**Neutrophils 10*9/L**	**Platelets 10*9/L**	**ASAT/ALAT U/L**	**Triglycerides mg/dL**	**Ferritin**	**Fibrinogen g/L**
1	81	0.55	25	125/203	262	5093	3.9
2	76	0.4	5	115/156	560	21994	0.88
3	74	1.1	27	27/34	235	1664	3.34
4	68	0.11	25	2200/2920	140	NR	0.2
5	71	0.15	44	4350/1990	252	NR	0.77
6	103	1.1	5	243/173	256	45	1.4
7	139	0.45	142	697/63	700	715	2.61
8	93	0.17	39	763/ 171	333	867	0.66
9	78	0.05	18	32/ 22	91	NR	3.6
10	88	1.02	81	50/26	428	1518	3.64
11	93	2.9	22	1163/2374	171	NR	1.05
12	86	2.03	40	22/28	158	87	2.34
13	92	1.8	38	46/48	188	709	2.95
14	78	0.32	101	112/112	234	NR	3.63
15	78	1.64	115	43198	295	NR	4.91
16	86	1.03	21	57/208	268	NR	1.68
17	98	2.1	101	261/367	333	7876	2.82
18	84	1.32	47	712/18	187	NR	3.02
19	76	1.53	50	150/ 227	251	NR	4.37
20	71	0.1	6	43444	486	NR	2.01
21	73	1.84	172	14/22	259	NR	3.63
22	109	2.4	380	27/55	303	740	2.55
23	92	4.5	273	22/18	165	208	5.32
24	85.5	0.1	22	307/49	900	10009	1.19
25	69	0.045	18	2209/1461	725	NR	0.73
26	73	0.75	44	34/34	230	NR	6.63
27	84	0.14	13	18/24	307	NR	0.77
28	89	3.92	104	47300	285	2454	2.19
29	84	1.5	30	34/29	205	2666	2.73
30	69	0.4	5	38/24	618	6195	2.23
31	94	0.1	62	232/253	478	31787	1.32
32	84	1.6	23	36/93	235	2969	2.88
33	29	1.4	63	104/59	498	8271	2.19

ASAT- aspartate aminotransferase
ALAT- alanine aminotransferase
NR not reported

The biopsy images were uniformly presented by benign histiocytes hyperplasia, independently of the associated infections or co morbidities. In nine of those cases, the haematopoietic bone marrow cellularity was significantly erased by the histiocytes hyperplasia. The haemophagocytosis was a common event in each biopsy and in some cases accompanied by cellular debris.

Outcomes

Table 3 Overall mortality: 19 (57.6%) patients from the 33 in our study died (overall mortality rate 57.6%). The average period from time of hospitalisation to death was 49.95 days (49.95+/-39.608), range 14-180 days. The overall mortality for the patients with underlying autoimmune disorder (MAS) was lower, 36.3% (4 patients died and six survived). The consecutive monitoring of one of those patients was lost after one year. According to time of death, 7 patients (21.2%) died in less than one month, and 15 (45.5%) died in less than two months. Only one patient with MAS died in less than one month, while the remaining patients had a higher rate (six). Risk factors significantly associated to overall mortality were age >35.08 (p<0.009), underlying

Table 3. Overall mortality

Prognostic factors	P Overall mortality	P Extremely severe disease	P Severe disease
Age (Equal variances): Set 1 age 51.47 (19 died). Set 2 age 35.08(11 survived)	0,009	NS	NS
Gender	NS	NS	NS
Autoimmune disease(n=11)	NS	NS	0.003; Odds 0.057(0.006-0.532)CI 95%
Solid organ cancer (n=5)	NS	0.043 Odds 10.000(0.754-132.682) CI95%	NS
Underlying infection (n=23)	NS	NS	0.053 Odds 5.200 (0.899-30.078)CI95%
Underlying treatment with corticosteroids alone (n=6)	NS	NS	0.013 Odds 2.250(1.476-3.430)CI95%
Underlying treatment with Mycophenolate mofetil (n=4)	0.028 Odds 2.900(1.756-4.789)CI95%	NS	0.051 Odds 2.071(1.421-3.019)CI 95%
Severe anaemia (n=19)	0.002 Odds 12.500(2.290-68.245)CI95%	NS	0.002 Odds 13.000(2.187-77.266) CI95%
Severe neutropenia (n=13)	NS	NS	0.027Odds 5.250(1.151-23.937)CI95%
Severe thrombocytopenia (n=15)	NS	0.016 Odds 11.333(1.176-109.256) CI95%	NS
Severe anaemia and thrombocytopenia(n=11)	NS	0.016 Odds 8.333(1.276-54.423)CI95%	NS
Severe anaemia and neutropenia (n=9)	0.007 Odds 2.182(1.412-3.371) ci95%	0.046 Odds 5.600(0.938-33.428) CI95%	0.002 Odds 19.429(2.032-185.724) CI95%
Severe pancytopenia(n=7)	0.025 Odds2.000(1.362-2.937)CI95%	0.009 Odds 10.222(1.498-69.761) CI95%	0.016 Odds 11.333(1.176-109.256) CI95%
Severe hypofibrinogenemia (n=6)	NS	0.057 Odds 5.750(0.843-39.241)CI95%	0.039 Odds 8.500(0.865-83.493)CI 95%
Lung involvement n(=7)	0.012 Odds 7.222(1.440-36.224)CI95%	NS	0.022 Odds 5.500(1.219-24.813)CI95%
Ongoing sepsis (n=6)	0.044 Odds 1.929(1.341-2.774)CI95%	NS	NS
Without specific treatment (n=3)	NS	0.043 Odds 10.000(0.754-132.682)CI95%	0.047 Odds 2.500(1.613-3.875)CI 95%
Autoimmune diseases with immunosuppresants at the onset(n=10)	0.061 Odds 0.219(0.42-1.135)CI95%	NS	P<0.007 Odds 0.71(0.008-0.664)CI95%

NS=not significant

treatment with MFM (p<0.028), severe anaemia (p<0.007), severe bicytopenia (anaemia and neutropenia) (p<0.007), severe pancytopenia (p<0.025), ongoing lung involvement (p<0.012) and severe ongoing infection or sepsis (p<0.044). Risk factors for extremely severe disease were underlying solid organ cancer (p<0.043), severe thrombocytopenia (p<0.016), severe bicytopenia such as anaemia and thrombocytopenia (p<0.016), and anaemia with neutropenia (p<0.046), severe pancytopenia (p<0.009) and a late start to specific treatment (p<0.043). Severe hypofibrinogenemia (<1.0 g/L) showed a trend towards a significance (p<0.057) for extremely severe disease. Risk factors for severe disease were underlying treatment with alone (p<0.013), severe anaemia (p<0.002), severe neutropenia (p<0.027) and severe pancytopenia (p<0.016). Severe hypofibrinogenemia (p<0.039), ongoing lung involvement (p<0.022) and a late start to specific treatment (p<0.047) were highly indicative of severe disease. Infection at the onset showed a trend towards a significance for severe disease (p<0.053).

Patients with MAS had a better prognosis (p<0.003) and the simultaneous use of immunosuppressant typical for those patients, seemed to act as a protective factor (p<0.007), corresponding odds ratios (OR)=0.057(0.006-0.532) CI 95%, and Odds 0.71(0.008-0.664) CI 95%. Statistically significant outcomes for variables such as gender, splenomegaly, haepatopathy, ferritin or triglyceride levels as well as treatment strategies 2, 3 or 4 were not found.

Discussion

HLH is a rare, potentially life threatening immune disorder which includes a long lasting fever, haepatoslnomegaly and laboratory findings such as cytopenias, hyperferritinaemia, hypertriglyceridemia and/or hypofibrinogenaemia. Pathogenesis remains unknown; nevertheless, impaired T and natural killer (NK) cell cytotoxicity seem to be involved [5]. Over the last few decades a variety of genetic disorders have been discovered in patients with FHLH, all responsible to encode proteins participating in the granule-dependent cytotoxic function of natural killer (NK) and T cells [6]. All cytotoxic cells are equipped with cytotoxic granules, called lysosomes, where perforin and granzym B are stored. Upon activation of natural killer (NK) cells and cytotoxic lymphocytes (CTLs), perforin is inserted into the plasma membrane and starts polymerisation. This results in perforin forming pores in the cell membrane and granzym B enters the target cell, giving the start signal for the programmed cell death [7]. The presence of defects in the granule dependent cytotoxic activity may result in that NK and CTLs fail to kill target cells and remove the source of antigenic stimulation. The persistent antigenic stimulation leads to continuous antigen driven activation and proliferation of new cytotoxic T cells with overproduction of inflammatory cytokines, such as interferon γ (IFR γ). In response to this continuous stimulation, macrophages become haemophagocytic. In 2010, Grom and Mellins showed that cytolytic function in patients with MAS was found to be profoundly depressed [8]. Low NK cytolytic activity has been also reported in patients with SJIA, and that data was used to differentiate those patients from the other forms of childhood arthritis. It appears that multiple factors; genetic abnormalities and acquired factors might lead to abnormal cytolytic function in patients with SJIA and MAS. Later, in 2012, Canna and Behrens presented IFN gamma as a critical pro-inflammatory mediator, which delivered via osmotic pump is sufficient to cause haemopahgocytosis and anaemia [9]. The same reviewers described the mechanisms by which the combination of viral infection and abnormal cytolytic function might result in HLH, suggesting that MAS and HLH are overlapping diseases.

An important finding in our study is that, that patients with MAS have a lower risk of severe disease. To date, several studies have confirmed that data Table 4. Dhote, in a study of twentysix cases with underlying autoimmune disorder found overall mortality 38.5% [10]. Fukaya, in a study of 30 similar cases reported overall mortality 20% [11]. Takahashi, in a study of 52 cases (including lymphoma, virus, bacteria and autoimmune associated HLH) showed that prognosis depended on the underlying disease and found that patients with lymphoma associated HLH had a lower survival rate [12]. Otroch, in a similar study of 73 cases (including Infection, malignancies, primary immunodeficiency, post solid organ transplantation, idiopathic and autoimmune associated HLH) pointed out malignancy as a risk factor [13]. Our study confirmed that patients with underlying solid organ cancer had a worst prognosis. We also discovered that patients with MAS and those who had simultaneous immunosuppressive

Table 4. Risk of severe diseases in MAS patient

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Authors and ref.	**Underlying pathology**	**Mortality**	**Time to death (days)**	**Overall age**	**Prognostic factors**
Dhote [10] (n=26)	SLE, RA, ASD, Poliarteritis nodosa, mixed connective- tissue disease, pulmonary sarcoidosis, systemic sclerosis, SS	38.50%	NR	47	Adenomegaly, corticosteroids or immunosuppressant at the onset, thrombocytopenia
Fukaya [11] (n=30)	SLE,RA,polymyositis/ dermatomyositis, systemic sclerosis, vasculitis, SS, ASD	20	NR	43	Age>50 years, infection or high CRP level, leukopenia, thrombocytopenia
Takahashi N [12] (n=52)	Malignant lymphoma, SLE, viral infections, bacterial and fungal infections	88 L* 12 NL*	83 L* 44 NL*	NR	Underlying disease, anaemia, thrombocytopenia, increased ferritin
Otrock Z.K [13] (n=73)	Infection, malignancies, autoimmune disorders, primary immunodeficiency, post-solid organ transplantation, ideopatic	48	NR	51	Malignancy, male gender, age>30, renal insufficiency, ferritin level >50.000 mg/l
Karras A [14] (n=17)	Renal transplantation	47	51	41	Organomegaly, elevated aminotransferase levels, abnormal prothrombin time, thrombocytopenia
Shabbir M [15] (n=18)	Haematological malignancies, bone marrow transplant, SLE, ASD, gram(-) sepsis, liver transplants, sickle cell disease	67	37	56	Fever
Warley [16] ((n=27)	Immunosuppressant therapy, solid organ cancer, infection, unknown	53.4	33	51	Renal failure, treatment of underlying disease acts as a protective factor
Kaito K. [17] (n=34)	Haematological malignancies, viral infections, unknown	NR	NR	44	Age >30 years, disseminated intravascular coagulation (DIC), increased ferritin, increased beta 2-microglobullin, anaemia, thrombocytopenia, jaundice

NR- not reported, L*- lymphoma associated HLH, NL*- non lymphoma associated HLH

treatment seemed to have less severe disease. That data was not confirmed by other studies where underlying immunosuppressive treatment was more likely associated with a worst prognosis Table 4. Factors such as younger age and lower association with comorbidities might act as biases. Future studies are necessary in order to clarify that data.

Overall mortality in our study was higher (57.6%) and closer to studies focused on malignancies, virus and post transplantation associated HLH. Karras, in a study of 17 patients with renal transplantation associated HLH reported overall mortality 47% [14]. Shabbir, in a study of 18 patients with haematological malignancies, post-transplantation, sepsis, and autoimmune diseases associated HLH reported 67%, and Warley, in a similar study, 53.4% [15,16]. Our study highlighted that younger patients (<35.08 years) had a better survival rate. This data was confirmed by several authors. In 1997, Kaito reported age >30 years as a significant factor for the patients survival [17]. In 2008, Fukaya pointed out age >50 years and later, in 2015, Otrock, age >30 years associated with a worst prognosis Table 4. Cytopenia involving >2 cell lines was defined as a diagnostic criterion according to Haemophagocytic Limphohistiocytosis guidelines 2004. Over the last 10 years, several authors pointed out bicytopenia, usually attributed to hypercitokinemia, as important factor for the patient's survival. Lehmberg, in

a review of sensibility and specificity of HLH guidelines 2004 exposed that bicytopenia was fulfilled in most cases and neutrophils were frequently the last cell line to drop [18]. Our study found a close relationship between severe anaemia, bicytopenia and pancytopania and patient survival. Such data was confirmed by a wide variety of studies. Takakashi and Kaito reported anaemia and thrombopenia, Fukaya, leucopenia and thrombocytopenia, and Dhote, thromcytopenia related to a poor prognosis Table 4. The excess of ferritin usually reflects macrophage activation and suggests that high levels of ferritin, and more important, a failure of ferritin to fall dramatically after specific treatment, highly indicative of a worse prognosis. Unfortunately, our study did not demonstrate a significant relationship between ferritin levels and prognosis, probably because of the low sampling size. Nevertheless, that data was confirmed by authors such as Takakashi, Otrock and Kaito Table 4. Hypofibrinogenemia is not always present at the beginning of HLH, even if in some cases it could be a normal fibrinogen level. This abnormality usually is due to the fibrinogen property to act as acute phase protein. However, a normal fibrinogen range in patients with long lasting fever should be suspicious. Our study highlighted severe hypofibrinogenemia (fibrinogen levels <1.0 g/L) as an important risk factor for a worst prognosis and that date was confirmed by Karras and Kaito Table 4. Underlying treatment with corticosteroids and/ or immunosuppressant was reported by Dothe as a risk factor for a worst prognosis Table 4. In our study, we tried to analyse that data and we confirmed that patients who had received corticosteroids alone had a worse prognosis. Having an infection at the onset was found statistically significant for patient´s survival. Infection and/ or high C reactive protein levels, as risk factors for poor prognosis were reported by Fukaya Table 4. We tried to analyse that data and we found that underlying infection showed a trend towards a significance for a worse prognosis. Furthermore, we separately analysed patients with ongoing infection or sepsis and we found strong relationship with mortality. Our study also discovered that patients with ongoing lung involvement had a higher mortality rate. Such data has not been analysed by other authors and should be cautiously interpreted because of the fact that patients with lung involvement usually have a severe ongoing infection. Micophenolate mofetil (MFM) as a possible cause of haemophagocytic syndrome was mentioned by Raffray in 2010 [19]. We analysed four patients with underlying MFM treatment and obtained similar results. Nevertheless, such data should be cautiously interpreted because of the small sampling size and other confounding factors.

Therapeutic strategy: HLH treatment remains challenging

Suppression of the severe hyper inflammation and reduction of the storm of cytokines is the main and immediate aim of HLH treatment. Identifying triggering infections and elimination of the stimulus for the rapid but ineffective T-cell activation is the second but not less important point in this management. The first achievement in the treatment of HLH in adults has been reported in 2 protocols, the HLH-94, and the HLH-2004 [20]. Both recommend the use of etoposide in combination with corticosteroids and cyclosporine A (CSP) as a successful treatment to achieve remission. Corticosteroids, which are cytotoxic for lymphocytes and inhibit cytokines expression, are one of the most important therapeutic agents [21]. Dexamethasone (DXA) crosses the blood brain barrier better than prednisolone and is the preferred glucocorticoid for less severe cases [22]. CSP, which inhibit T lymphocyte activation, and immunoglobulin infusions (IVIG), which act by providing cytokines and pathogen-specific antibodies, are also frequently used. IVIG could be successfully used if started early in HLH treatment [23]. Etoposide is an effective drug for monocytic and histiocytic disease usually preserved for more severe cases. Etoposide has also been reported as a lifesaving, especially in patients with EBV associated HLH [24]. The HLH 94 protocol should not be used at all in patients with MAS where methylprednisolone pulse for 3 days, followed by methylprednisolone 2-3 mg/kg is the preferred drug [25]. Recently the interleukin-1 receptor antibody Anakinra has been successfully applied in patients with MAS, showing promising results [26]. Several case reports describe the use of Rituximab (anti cd-20 monoclonal antibody) in patients with HLH secondary to Hodgkin lymphoma [27]. Patients with MAS were also successfully treated with Rituximab [28,29]. Drugs such as Alemtuzumab, infliximab, daclizumab and haematopoietic cell transplant have been also used as salvage therapies. Remission of the triggering disease might increase efficiency of immunosuppressive

therapy [30]. Anecdotal case with successful MMF treatment has been recently reported [31].

However, despite the fact that prognosis of HLH has improved over the last 10 years; it is essential not to delay specific treatment [32]. Our study demonstrates that early recognition and specific treatment is essential for the patient's survival. Unfortunately, we did not found relationships between the use of corticosteroids, CSP or Etoposide and prognosis. However, we confirmed that tight suspicion from the beginning remains lifesaving, and the use of new strategies might change the disease prognosis.

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